Host range of *Phytophthora* sp. causing leaf fall disease of nutmeg (*Myristica fragrans* Houtt.)

Sumbula, V.*, Sally K. Mathew and Mini Raj N.1

Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Thrissur-680 656, Kerala, India.

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ABSTRACT

Leaf fall disease caused by *Phytophthora* sp. has become a serious problem in major nutmeg growing areas of Kerala during South - West monsoon period. Host range study was carried out to find out the infectivity of *Phytophthora* isolates of nutmeg on other known hosts of *Phytophthora* spp. including arecanut, coconut, cocoa, rubber, black pepper, cardamom, camboge, vanilla, rose, coreopsis, eucalyptus, colocasia and citrus. *Phytophthora* isolates of nutmeg showed characteristic symptoms on rubber, vanilla, rose, coreopsis, eucalyptus and citrus, whereas, cocoa, black pepper and colocasia developed hypersensitive reaction and the pathogen failed to cause infection on arecanut, coconut, cardamom and camboge.

Key words: Host range, Leaf fall disease, Nutmeg, *Phytophthora*.

INTRODUCTION

Nutmeg, an evergreen tree spice, popular for its flavouring and therapeutic properties. It plays a considerable role in India’s agricultural export. Ernakulam, Thrissur, Idukki and Kottayam are the major nutmeg growing districts in Kerala (GOK, 2015). Recently, a leaf fall disease caused by *Phytophthora* sp. has become a serious problem in the state. The first authentic report of leaf fall of nutmeg due to *Phytophthora* sp. was from Kerala (Mathew and Beena, 2012). The causative organism, *Phytophthora*, is a destructive pathogen known to have very wide host range and it varies from species to species (Lamour et al., 2007) and causing diseases in several economically important spices and plantation crops in Kerala viz. black pepper, cardamom, vanilla, rubber, cocoa, coconut and arecanut. An important aspect in the continuity of a disease is the host range of the pathogen. Therefore, the present study was taken up to find out whether *Phytophthora* sp. of nutmeg can infect the already reported hosts of different *Phytophthora* species in Kerala.

MATERIALS AND METHODS

Collection of samples and isolation of the pathogen:

Diseased samples were collected from different locations of Thrissur (Parakkadavu, Kodissery, Poovathussery, Mambra, Venoor, Koottala and Mookkcanoor), Ernakulam (Kalady, Mattur, Thevarmadom, Sreemoolanagaram, Tharuvoor, Kanjoor, Desam and Koottattukulam) and Kottayam (Vaikom and Kuravilangad) districts of Kerala. The pathogen was isolated from infected nutmeg leaves and fruits, showing characteristic symptoms (Development of dark brown water soaked lesions mainly on the midrib of the leaves which later enlarged and spread along the lateral veins resulting in blighting. Petioles of the infected leaves showed black discoloration and resulted in premature defoliation). Isolation was carried out by tissue isolation method and pure cultures of the isolates were incubated in sterile water at 22±1°C (Ko, 2003).

Inoculation was done with zoospore suspension (10^6 spores ml^-1) of each of medicinal plant (eucalyptus), tuber (colocasia) and fruit crop (citrus) were artificially inoculated with *Phytophthora* isolate of nutmeg to find out its host range . Inoculation was carried out under lab condition on detached leaves and under *in vivo* condition on three month old seedlings with the most virulent isolate and 18 isolates obtained from different locations.

In vitro condition

Artificial inoculation was carried out with zoospore suspension of the most virulent isolate (isolate from Parakkadavu) and culture disc of all the 18 isolates of the pathogen on the detached leaves.

a) Zoospore inoculation: Detached leaves of selected host plants were surface sterilized with one per cent sodium hypochlorite solution and washed with three changes of sterile water and wiped with blotting paper. These leaves were kept separately in large Petri dishes contained sterile moistened blotting paper. A drop of zoospore suspension of

*Corresponding author’s e-mail: sumbulav101@gmail.com
1Department of Plantation Crops and Spices, College of Horticulture, Agricultural University, Thrissur-680 656, Kerala, India.
the most virulent isolate was placed on the upper surface of the leaves after pinprick injury and proper humidity (70±10%) was maintained for symptom development.

b) Culture disc method: Eight millimetre culture disc of different isolates of the pathogen from five day old culture was inoculated on the leaves of the various hosts. Inoculated area was covered with moistened cotton and kept in moistened poly bags.

Under in vivo condition: Seedlings of selected host plants were inoculated with the culture disc of the most virulent isolate on the lower leaf surface. Seedlings were covered with the moistened polythene bags and observations on symptom development were recorded at 24 h intervals.

RESULTS AND DISCUSSION

Eighteen diseased samples were collected from seven locations of Thrissur, eight locations of Ernakulam and two from Kottayam district, of which, sixteen were leaf and two were fruit samples. On isolation on carrot agar medium, the pathogen associated with the disease was found to be Phytophthora sp. based on cultural and morphological characters.

Host range study was carried out to find out the infectivity of Phytophthora isolates of nutmeg on other known hosts such as arecanut, coconut, cocoa, rubber, black pepper, cardamom, camboe, vanilla, rose, coreopsis, eucalyptus, colocasia and citrus and the symptoms developed on these selected hosts were observed and symptoms were same under both lab and in vivo conditions. The susceptible hosts showed characteristic symptoms of Phytophthora described in that particular host or that of symptoms observed on nutmeg. There was no variation in symptoms produced among the eighteen isolates. Of the 13 hosts tested only rubber, vanilla, rose, coreopsis, eucalyptus and citrus were found susceptible to Phytophthora isolate of nutmeg by producing typical symptom of dark brown water soaked lesions on the midrib of leaves which later spread to leaf lamina and resulted in blighting (Table 1). In cocoa, black pepper and colocasia symptom developed as necrotic lesions showing hypersensitive reaction and the crops arecanut, coconut, cardamom and camboge did not show any symptoms. Further conformation with zoospore suspensions of 18 isolates on the detached leaves of these host under lab condition also yielded same result and all isolates showed same type of symptom of that particular susceptible host.

Symptoms on rubber: Initial symptom was developed after 24 h of inoculation with culture disc and 10 days with zoospore suspension. It showed typical symptoms of abnormal leaf fall of rubber caused by P. meadii. Symptom appeared as light brown discoloration on the midrib which later turned to dark brown water soaked lesion and spread along the lateral veins to leaf lamina. Coagulated latex was also observed on the midrib. Another characteristic symptom was the bronzing of the young foliage. Petioles of the infected leaves showed black discoloration resulted in premature defoliation of leaves in three days after inoculation (Figure 1). Symptoms on vanilla: Initial symptom was observed at 2 and 11 DAI by culture disc and zoospore inoculation methods respectively. The symptom appeared as black water soaked lesion which enlarged and spread to entire leaf lamina resulted in rotting of the leaves (Figure 2).

Symptoms on eucalyptus: Initial symptom developed 2 and 11 DAI by culture disc and zoospore inoculation methods respectively. The symptom first appeared as black water soaked lesions on the midrib of leaves, which enlarged and spread along the vein and veinlet to entire lamina and no defoliation was noticed (Figure 3).

Table 1: Host range of Phytophthora isolate of nutmeg

<table>
<thead>
<tr>
<th>Host</th>
<th>Symptom</th>
<th>Days for infection</th>
<th>Days for leaf fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutmeg (control)</td>
<td>Dark brown water soaked lesion on the midrib of the leaves, enlarged &amp; spread to lamina resulted in blighting, premature defoliation - Characteristic symptom of Phytophthora disease</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Arecanut</td>
<td>No infection</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coconut</td>
<td>No infection</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cocoa</td>
<td>Small dark brown necrotic lesion with yellow halo - Hypersensitive reaction</td>
<td>3</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Rubber</td>
<td>Characteristic symptom of P. meadii on rubber</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Black pepper</td>
<td>Hypersensitive reaction</td>
<td>3</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Cardamom</td>
<td>No infection</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Camboe</td>
<td>No infection</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Characteristic symptoms of P. meadii on vanilla</td>
<td>2</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Rose</td>
<td>Characteristic symptoms of P. ramorum on rose</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Coreopsis</td>
<td>Characteristic symptoms of Phytophthora disease of nutmeg</td>
<td>2</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Colocasia</td>
<td>Hypersensitive reaction</td>
<td>4</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Characteristic symptoms of Phytophthora on eucalyptus</td>
<td>2</td>
<td>No leaf fall</td>
</tr>
<tr>
<td>Citrus</td>
<td>Characteristic symptoms of P. citrophthora on citrus</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
**Symptoms on rose:** Initial symptom was noticed at 1st day after inoculation with culture disc method. Symptom appeared as dark brown water soaked lesion at the midrib which spread along the lateral veins and leaves turned yellow and defoliated at 3 DAI.

With zoospore inoculation, symptom appeared 5 DAI as water soaked lesion on the leaves and petiole. Both green and yellow leaves defoliated at 7 DAI. Water soaked lesion was also observed on buds led to rotting, which spread to flower stalk and resulted in shedding of buds (Figure 4).

**Symptoms on coreopsis:** Infection started at two days after inoculation as black water soaked lesion on the inoculated area, which later spread along the veins and veinlets to entire lamina causing rotting of the leaves (Fig.5).

**Symptoms on citrus:** The symptom first appeared a slight brown water soaked lesion on the midrib of leaves at 2 DAI which enlarged and spread along the veins and veinlets and resulted in degreening of the leaves. Infected leaves defoliated on 4 DAI(Fig.6).

**Symptoms on cocoa, black pepper and colocasia:** Infection initiated at 3 DAI in cocoa and black pepper and 4 days in *Colocasia* as small black lesion with yellow halo, which later became necrotic without further spread, showing hypersensitive reaction(Fig.7-9).
The pathogen could be reisolated from the hosts which developed water soaked lesion and showed typical characters of the original culture and the pathogenicity could be proved. However, the reisolation of the pathogen from the hosts which showed hypersensitive reaction, failed to produce any fungal growth on the media.

Thus the findings in the present revealed that, the *Phytophthora* isolate of nutmeg was found to have host range, including rubber, vanilla, rose, coreopsis, eucalyptus and citrus. Prem(1995) reported coconut, rubber, black pepper and colocasia as the hosts of *P. palmivora* of cocoa. Similarly, Sarma *et al.* (2003) reported rubber, cocoa, arecanut, cardamom and vanilla as the hosts of *P. meadii*. *P. ramorum* attacks different cultivar of *Rosa* spp. (Moralejo& Hernandez, 2002), *Eucalyptus* (Brasier *et al.*, 2005), *Citrus limon* and *Citrus deliciosa* (Moralejo *et al.*, 2006). Singh (2012) identified colocasia, rubber, and black pepper as hosts of *P. colocasiae*.

**CONCLUSION**

The present study revealed that, of the 13 hosts screened, six namely, rubber, vanilla, rose, coreopsis, eucalyptus and citrus were found to be susceptible to *Phytophthora* of nutmeg and showed the characteristic symptom, as observed on nutmeg leaves. It is also observed that, the infection of *Phytophthora* of nutmeg on rubber and vanilla showed characteristic symptoms as that of abnormal leaf fall and Phytophthora rot caused by *P. meadii* respectively. Plants such as cocoa, black pepper and colocasia developed necrotic hypersensitive reaction and the pathogen failed to cause infection in arecanut, coconut, cardamom and camboge. The findings in the present revealed that, the *Phytophthora* isolates of nutmeg were found to have host range, including rubber, vanilla, rose, coreopsis, eucalyptus and citrus and these hosts can also serve as a collateral hosts, for the perpetuation of the pathogen.

**REFERENCES**


